Remarks

Based on the amendments to the claims and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

I. Status of the Claims

Claims 1, 2, 4, 5, 9-25, 28-41, 43-56, and 58-70 are under consideration of which claims 1, 53, and 54 are independent. Claims 5, 9, 10, 53, and 54 are amended with this response. Claims 5, 9, and 10 have been amended to change their dependency. Support for these amendments can be found throughout the specification, *inter alia*, in the originally filed claims. Support for the amendments to claims 53 and 54 may be found throughout the specification, *inter alia*, at page 6, lines 21-23, page 7, lines 3-4, and in the Examples. No new matter has been introduced by these amendments.

Applicants note that claims 15 and 39 were not rejected nor were they indicated as allowable if re-written in independent form. Clarification of the status of these claims is requested.

II. Summary of the Office Action

In the Office Action dated May 8, 2002, the Examiner made 9 rejections of the claims. Applicants respectfully offer the following remarks to overcome these rejections.

III. The Rejection of Claims 5, 9, 10 and 54 Under 35 U.S.C. § 112, Second Paragraph, Must be Withdrawn

In the Office Action at page 2, section 3, claims 5, 9, 10, and 54 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. Applicants respectfully request reconsideration and withdrawal of this rejection.

Claim 5 was alleged to be indefinite as depending from cancelled claim 3. Claim 5 has been amended to depend from currently pending claim 4. Applicants respectfully submit that this amendment obviates the rejection of claim 5 and respectfully request reconsideration and withdrawal of this rejection.

Claim 9 was alleged to be indefinite as the phrase "the surface" lacked antecedent basis. Claim 9 has been amended to depend from claim 2, which provides antecedent basis for "the surface." Applicants respectfully submit that this amendment obviates the rejection of claim 9 and respectfully request reconsideration and withdrawal of this rejection.

Claim 10 was alleged to be indefinite as the phrase "the fixative" lacked antecedent basis. Claim 10 has been amended to depend from claim 2, which provides antecedent basis for "the fixative." Applicants respectfully submit that this amendment obviates the rejection of claim 10 and respectfully request reconsideration and withdrawal of this rejection.

Claim 54 was alleged to be indefinite for the use of the phrase "characterizing a single cell environment." The Examiner alleges that this phrase is indefinite as it does not specify an intracellular environment or extracellular environment. The phrase "single cell environment " is defined in the specification at page 7, line 4, to mean "a single cell or a group of cells isolated from one source." In view of this definition, Applicants respectfully submit that this phrase is not indefinite and respectfully request reconsideration and withdrawal of this rejection.

Claim 54 was alleged to be indefinite for failing to recite an action to be taken after a concurrent measurement. Claim 54 has been amended to read "A method of establishing a characterization profile of a circulating epithelial cancer cell obtained from a body fluid comprising characterizing a single cell environment by concurrent measurement ... ." Applicants respectfully submit that this amendment obviates the rejection of claim 54. Applicants respectfully request reconsideration and withdrawal of this rejection

IV. The Rejection of Claims 1, 2, 4, 5, 9, 10, 11, 12, 33-38, 40, 41, 43-46, 51, 53-56, 59, and 62-70 Under 35 U.S.C.§ 102(a) As Allegedly Being Anticipated by Wang, et al. Must Be Withdrawn

In the Office Action at page 3, section 5, claims 1, 2, 4, 5, 9, 10, 11, 12, 33-38, 40, 41, 43-46, 51, 53-56, 59, and 62-70 have been rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Wang, *et al.*, (International Symposium on Biology of Prostate Growth, March 1998, document AS18 of the IDS filed 2/15/2002, hereinafter "Wang"). Applicants respectfully request reconsideration and withdrawal of this rejection.

35 U.S.C. § 102(a) reads:

> A person shall be entitled to a patent unless
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign, before the invention thereof by the applicant for patent,

Applicants respectfully submit that the invention described in Wang is the Applicants' own work and the other co-authors did not make any inventive contribution. Applicants submit herewith a Declaration signed by inventor Paul O.P. Ts'o attesting to this fact. Accordingly, Wang is not available as a reference against this application. In

view of the foregoing, Applicants respectfully request reconsideration and withdrawal of this rejection.

During the course of preparing the Declaration referred to above, it was determined that Zheng-Pin Wang is not an inventor of the presently claimed methods. Accordingly, Applicants are preparing a Change-of-Inventorship that will be submitted in this application to the effect that only Stephen A. Lesko and Paul O.P. Ts'o are the inventors of the claimed invention.

V. *The Rejection of Claims 1, 11, 12, 13, 14, 16, 17, 37, 38, 40, 41, 45, 46, 60, 62, and 70 Under 35 U.S.C. §102(b) As Allegedly Being Anticipated by Shackney, et al. Must Be Withdrawn*

In the Office Action at page 6, section 6, claims 1, 11, 12, 13, 14, 16, 17, 37, 38, 40, 41, 45, 46, 60, 62, and 70 have been rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Shackney, *et al.* (*Cytometry*, 1995, Vol. 22, pp. 282-21, hereinafter "Shackney"). Applicants respectfully request reconsideration and withdrawal of this rejection.

A claimed invention is anticipated under 35 U.S.C. § 102 only if there is "[d]isclosure in a single piece of prior art of each and every limitation of a claimed invention." *Apple Computer, Inc. v. Articulate Systems, Inc.*, 234 F.3d 14, 20, 57 USPQ2d 1057, 1061 (Fed. Cir. 2000), *citing Electro Med. Sys. S.A. v. Cooper Life Sciences,* 34 F.3d 1048, 1052, 32 USPQ2d 1017, 1019 (Fed. Cir. 1994). Shackney does not disclose the invention as presently claimed. Accordingly, this rejection is improper and must be withdrawn.

Claims 1, 53, and 54 are independent claims. Claims 11, 12, 13, 14, 16, 17, 37, 38, 40, 41, 45, and 46 depend—directly or indirectly—from claim 1. Claims 60, 62, and

70 depend from any one of claims 1, 53, or 54. Claim 1 is drawn to a method of characterizing *single circulating epithelial cancer cells* obtained from a body fluid comprising the concurrent measurement of multiple cellular markers using fluorescent probes, wherein said probes emit different wavelengths of light to distinguish multiple cellular markers expressed in said single cell using fluorescence microscopy. Independent claim 1 and, therefore, all of the rejected claims, require characterization of a circulating epithelial cancer cell. Shackney does not disclose characterization of a circulating epithelial cancer cell. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

VI. The Rejection of Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70 Under 35 U.S.C. § 103(a) As Allegedly Being Unpatentable Over Simpson, et al., Waggoner, et al., Galbraith, et al., Dale, et al., Frudakis, et al., and Gross, et al., Must Be Withdrawn

In the Office Action at page 6, section 7, claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Simpson, *et al.* (*Experimental Hematology* 23: 1062-1068, (1995) document AS9 of IDS filed 4/27/00, hereinafter "Simpson"), in view of Waggoner, *et al.* (*Human Pathology* 27(5): 494-502, (1996), Ref AT15 of the IDS filed 1/28/01, hereinafter "Waggoner"), Galbraith, *et al.* (*Cytometry* 12:579-596, (1991), document AS15 of the IDS filed 1/28/01, hereinafter "Galbraith"), Dale, *et al.* (*Proc. Annu. Meet. Am. Soc. Clin. Oncol.* 14: A1308, (1995), hereinafter "Dale"), Frudakis, *et al.* (United States patent number 6,344,550, hereinafter "Frudakis"), and Gross, *et al.* (*PNAS* 92:537-541, (1995), hereinafter "Gross"). Applicants respectfully request reconsideration and withdrawal of this rejection.

Claims 1 and 54 are independent claims. Claims 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 56, and 58 depend—directly or indirectly—from claim 1. Claims 60, 62 and 70 depend from any one of claims 1, 53, or 54. Claim 1 is discussed above. Claim 54 is drawn to a method of establishing a characterization profile of a circulating epithelial cancer cell obtained from a body fluid comprising characterizing a single cell environment by concurrent measurement of multiple cellular markers using fluorescent probes, wherein said probes emit different wavelengths of light to distinguish multiple cellular markers expressed in the single cell using fluorescence microscopy. All of the claims are to methods of characterizing single circulating epithelial cancer cells and require the use of fluorescence microscopy for concurrent measurement of multiple cellular markers using fluorescent probes.

As stated in the MPEP:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the references or to combine reference teachings. Second , there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

MPEP § 2143.

Applicants respectfully submit that, based on the combination of the cited documents, one of ordinary skill in the art would not have had a reasonable expectation of successfully making and practicing presently claimed invention. Accordingly, Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case for the obviousness of the presently claimed invention and respectfully request reconsideration and withdrawal of this rejection as it may be applied to the present claims.

The Examiner cites Simpson, alleging "Simpson et al. teach a method for identifying single circulating breast cancer cells in the blood of breast cancer patients . . . ." Applicants respectfully submit that this is a mischaracterization of the flow cytometry based method used by Simpson. Flow cytometry does not characterize single cells, flow cytometry characterizes populations of cells. Flow cytometry requires a large population of cells to produce statistically valid results. As can be seen in Figure 1A of Simpson, any single cell (*i.e.*, the dots in Figure 1A) produces a single data point that cannot be interpreted. In Simpson, it is only after accumulation of many data points that the entire population, not a single cell, can be statistically analyzed. The individual cells in Simpson are not characterized as called for in the present claims. Moreover, Simpson uses flow cytometry to identify cancerous cells and does not disclose the use of fluorescence microscopy as called for in the claims. Accordingly, Simpson is seriously deficient as a reference against the presently claimed invention.

The additional documents cited by the Examiner do not cure the deficiencies of Simpson. The Examiner cites Dale for the use of multiple markers to identify melanoma cells and Frudakis for the use of multiple markers for detecting breast cancer cells. Neither of these documents teaches the use of multiple fluorescent probes to detect multiple markers. Likewise, neither of these documents teaches the use of fluorescence microscopy to detect cancerous cells. Instead, Dale discloses an RT-PCR based method (see Abstract, second sentence) and makes no mention of the use of any fluorescent probes whatsoever. Although Frudakis discloses the production of antibodies against the tumor antigens identified, which antibodies may contain reporter groups (see column 9, line 49 to column 12, line 40), Frudakis does not disclose the use of multiple fluorescent probes that can be concurrently detected and does not disclose the use of fluorescence

microscopy to detect cancerous cells. Thus, neither of these two documents cures the deficiencies of Simpson.

The Examiner cites Gross, alleging Gross discloses the use of three different anti-cytokeratin antibodies labeled with three different labels. Gross characterizes the disclosed methods as follows:

> The basic approach should be to stain the subpopulation of the cells one does not want to detect with one color (the exclusion color) and stain the rare cells with one, two, or three of the remaining colors. Measuring more than three or four colors does not help discrimination since aberrant positive values are often correlated (such as autofluorescence).(Page 541, paragraph bridging columns)

Gross discloses three probes and teaches that this is the limit of the detection capability of the method since additional probes would not be expected to improve the assay. In addition Gross used a model system prepared by mixing a known amount of cancer cells with isolated peripheral blood mononuclear cells. Gross, page 538. Thus, Gross does not characterize circulating cancer cells obtained from a body fluid as called for in the present claims. Further, when Gross discusses the possibility of using his method on body fluid samples, he concludes that large amounts of sample would be required for this method and that samples of this size are not practical. Gross page 541, Discussion. Finally, Gross does not teach the use of fluorescence microscopy to detect cancerous cells.

The Examiner cites Galbraith, alleging "Galbraith et al teach that fluorescent imaging cytometry is superior to flow cytometry in that the method is not limited by the number of fluorophores which may be distinguished (page 592, first column second full paragraph)." The cell population Galbraith uses is derived from normal individuals (page 580, left column second full paragraph) and none of the probes used by Galbraith detects

cancer markers. Thus, Galbraith does not cure the deficiencies of Simpson because Galbraith does not disclose or suggest the use of fluorescence microscopy to detect cancer cells.

The Examiner cites Waggoner, alleging "Waggoner et al teach multicolor analysis of populations of single cells and a microscope slide surface containing cells (page 499, first column, "Multicolor Immunophenotyping"), wherein the cells were well separated from each other by quantitative fluorescent imaging (page 499, second column, last paragraph)." (Office Action, page 8) Waggoner is a review article in which reagents and instruments used in fluorescent imaging microscopy are discussed. The work referred to by the Examiner as disclosing single cells well separated on a slide is the work of Galbraith discussed above. (See reference 33). Waggoner does not add anything to the disclosure of Galbraith; thus, Waggoner does not cure the deficiencies of Simpson.

One of ordinary skill in the art would have had no reasonable expectation that combining the teachings of the cited documents would successfully produce a method of characterizing circulating cancer cells by fluorescence microscopy as presently claimed because there is no disclosure or suggestion in Galbraith or any of the other documents that fluorescence microscopy can be used for characterizing the rare circulating epithelial cancer cells. In contrast, the cells detected by Galbraith were normal cells, which are present at high concentrations. One of skill in the art would have had no reasonable expectation that fluorescence microscopy as disclosed in Galbraith would be sufficiently sensitive to detect rare cancer cells. One of skill in the art would have had no reasonable expectation that a combination of the methods of the cited documents would be successful in characterizing single circulating epithelial cancer cells by concurrent measurement of multiple cellular markers by fluorescence microscopy as called for in the

present claims. The Examiner is using impermissible hindsight based on the disclosure of the captioned application to maintain the obviousness rejection over the combination of these documents.

In view of the remarks presented above, Applicants respectfully request reconsideration and withdrawal of this rejection.

VII. The Rejection of Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70 and Claims 47, 49, 50 and 52 Under 35 U.S.C. §103(a) As Allegedly Being Unpatentable Over Simpson, Waggoner, Galbraith, Dale, Frudakis, Gross, Zhang, et al. and Kute, et al. Must Be Withdrawn

In the Office Action at page 9, section 8, claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70 and claims 47, 49, 50 and 52 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Simpson in view of Waggoner, Galbraith, Dale, Frudakis, and Gross as applied to claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, and 70 above, and further in view of the abstract of Zhang, *et al.* (*Chinese Journal of Surgery* 35:474-477, (1997), hereinafter "Zhang") and the abstract of Kute, *et al.* (*Cytometry*, 4:132-140, (1983), hereinafter "Kute"). Applicants respectfully request reconsideration and withdrawal of this rejection.

Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, and 70 have been discussed above. Claims 47, 49, 50 and 52 depend from claim 1.

As discussed above, the combination of Simpson in view of Waggoner, Galbraith, Dale, Frudakis, and Gross would not have provided one of ordinary skill in the art a reasonable expectation of successfully making and using the claimed invention. Zhang and Kute do not remedy the deficiencies of this combination.

Kute is alleged to disclose cytofluorometric assays for estrogen receptors while Zhang is alleged to teach the evaluation of breast cancer patients by determining the estrogen or progesterone receptor status of tumor tissues. (Office Action, page 10). As neither of these documents discloses or suggests the characterization of single circulating epithelial cancer cells by concurrent measurement of multiple markers using multiple fluorescent probes using fluorescence microscopy, these documents do not cure the deficiencies of the combination discussed above. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

VIII. The Rejection of Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70 and Claim 59 Under 35 U.S.C. § 103(a) As Allegedly Being Unpatentable Over Simpson, Waggoner, Galbraith, Dale, Frudakis, Gross, and Ferrari Must Be Withdrawn

In the Office Action at page 10, section 9, claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70 and claim 59 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Simpson in view of Waggoner, Galbraith, Dale, Frudakis, and Gross as applied to claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, and 70 above, and further in view of Ferrari, *et al.* (*Proc. Annu. Meet. Am. Assoc. Cancer Res.* 37:A1686, (1996), hereinafter "Ferrari"). Applicants respectfully request reconsideration and withdrawal of this rejection.

Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, and 70 have been discussed above. Claim 59 depends from any one of claims 1, 53, or 54.

As discussed above, the combination of Simpson in view of Waggoner, Galbraith, Dale, Frudakis, and Gross does not teach or suggest the presently claimed

invention and is not a proper combination of references. Ferrari does not remedy the deficiencies of this combination.

Ferrari is alleged to teach the detection of circulating metastatic prostate tumor cells using a PCR based assay. Since Ferrari neither discloses nor suggests use of fluorescence microscopy to perform the concurrent measurement of multiple markers using multiple fluorescent probes to detect the markers, Ferrari cannot cure the deficiencies of the combined references discussed above. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

IX. The Rejection of Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70, 59 and claims 47, 48, and 52 Under 35 U.S.C. §103(a) As Allegedly Being Unpatentable Over Simpson, Waggoner, Galbraith, Dale, Frudakis, Gross, Ferrari, and Takeda Must Be Withdrawn

In the Office Action at page 11, section 10, claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70, 59 and claims 47, 48, and 52 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Simpson in view of Waggoner, Galbraith, Dale, Frudakis, Gross and Ferrari as applied to claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70, and 59 above, and further in view of the abstract of Takeda, *et al.* (*Cancer* 77:934-940, (1996), hereinafter "Takeda"). Applicants respectfully request reconsideration and withdrawal of this rejection.

Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70, and 59 have been discussed above. Claims 47, 48, and 52 depend indirectly from claim 1.

Takeda discloses the analysis of prostate biopsies using a labeled antibody to the androgen receptor. The abstract of the paper provided with the Office Action does not indicate how the antibodies were labeled and detected.

Since Takeda neither discloses nor suggests use of fluorescence microscopy to perform the concurrent measurement of multiple markers using multiple fluorescent probes to detect the markers, Takeda cannot cure the deficiencies of the combined references discussed above. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

X. The Rejection of Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70 and Claims 53 and 55 Under 35 U.S.C. §103(a) As Allegedly Being Unpatentable Over Simpson, Waggoner, Galbraith, Dale, Frudakis, Gross, and Thomas Must Be Withdrawn

In the Office Action at page 12, section 11, claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70 and claims 53 and 55 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Simpson in view of Waggoner, Galbraith, Dale, Frudakis, and Gross as applied to claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, and 70 above, and further in view of Thomas, *et al.* (United States patent number 6,117,985, "Thomas"). Applicants respectfully request reconsideration and withdrawal of this rejection.

Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, and 70 have been discussed above. Claim 53 is drawn to a method of characterizing a single circulating epithelial cancer cell preparation obtained from a body fluid comprising adhering a circulating epithelial cancer cell preparation to be characterized onto a surface, fixing said cell preparation with a fixative solution, incubating said cell surface containing fixed cells with multiple probes directed to desired cellular markers,

wherein said multiple probes have the ability to fluoresce when excited at different wavelengths, and examining the cells by fluorescence microscopy for identification of positive cells for each selected cellular marker by concurrent measurement of multiple cellular markers, wherein said cancer cell preparation is isolated from a body fluid using a negative selection process. Claim 55 depends from claim 53.

Thomas is alleged to teach negative selection methods to enrich for non-hematopoietic tumor cells. Thomas cannot cure the deficiencies of the references discussed above. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

XI. The Rejection of Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70 and Claim 61 are Rejected Under 35 U.S.C. § 103(a) As Allegedly Being Unpatentable Over Simpson, Waggoner, Galbraith, Dale, Frudakis, and Gross, in view of Z'Graggen or Leather or Komeda Must Be Withdrawn

In the Office Action a t page 13, section 12, claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70 and claim 61 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Simpson in view of Waggoner, Galbraith, Dale, Frudakis, and Gross as applied to claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, and 70 above, and further in view of the abstract of Z'Graggen, *et al.* (*Pancreas* 15(4):463, (1997), "Z'Graggen"), or the abstract of Leather, *et al.* (*British Journal of Surgery* 80(6):777-780, (1993), "Leather"), or the abstract of Komeda, *et al.* (*Cancer* 75(9):2214-2219, (1995), "Komeda"). Applicants respectfully request reconsideration and withdrawal of this rejection.

Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, and 70 have been discussed above. Claim 61 depends from any one of claims 1, 53, or 54.

Z'Graggen is cited for the proposition that the presence of circulating pancreatic tumor cells correlates to the presence of metastasis in pancreatic cancer patients. Leather is cited for the proposition that detection of circulating tumor cells in colorectal cancer is indicative of metastasis. Komeda is cited for the proposition that the presence of circulating hepatocellular carcinoma cells in peripheral venous blood is indicative of metastasis. Only the title of Z'Graggen was provided with the Office Action. Leather and Komeda do not disclose or suggest using fluorescence microscopy for concurrent measurement of multiple markers using fluorescently-labeled probes, and thus fail to remedy the deficiencies of the cited documents discussed above. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

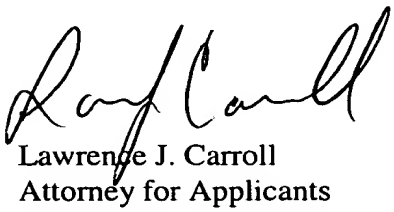
Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



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Version with markings to show changes made

The claims have been amended as follows:

5. (Amended) The method of claim [3] 4, wherein said body fluid is selected from the group consisting of blood, bone marrow, saliva, cerebrospinal fluid, urine, a body cavity fluid, and semen.

9. (Amended) The method of claim [1] 2, wherein the surface for cell adherence is a microscope slide.

10. (Amended) The method of claim [1] 2, wherein the fixative is selected from a group consisting of paraformaldehyde, formaldehyde, alcohol, or acetone.

53. (Twice amended) A method of characterizing a single circulating epithelial cancer cell preparation obtained from a body fluid comprising adhering a circulating epithelial cancer cell preparation to be characterized onto a surface, fixing said cell preparation with a fixative solution, incubating said cell surface containing fixed cells with multiple probes directed to desired cellular markers, wherein said multiple probes have the ability to fluoresce when excited at different wavelengths, and examining the cells by fluorescence microscopy for identification of positive cells for each selected cellular marker by concurrent measurement of multiple cellular markers, wherein said cancer cell preparation is isolated from a body fluid using a negative selection process.

54. (Twice amended) A method of establishing a characterization profile of a circulating epithelial cancer cell obtained from a body fluid comprising characterizing a single cell environment[, wherein the] by concurrent measurement of multiple cellular markers using fluorescent probes, wherein said probes emit different wavelengths of light to distinguish multiple cellular markers expressed in the single cell using fluorescence microscopy.